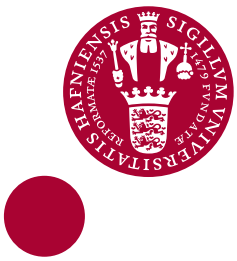

Low-fat fermented milk with a combination of fructooligosaccharides and live Lactobacillus rhamnosus GG (ATCC 53103), Streptococcus thermophilus (Z57) andLactobacillus bulgaricus (LB2), and defence against reactivation of Herpes simplex virus in the orolabial epithelia: evaluation of a health claim pursuant to Article 13(5) of Regulation (EC) No 1924/2006

(Scientific Opinion)

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)

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Low-fat fermented milk with a combination of fructo-oligosaccharides and live *Lactobacillus rhamnosus* GG (ATCC 53103), *Streptococcus thermophilus* (Z57) and *Lactobacillus bulgaricus* (LB2), and defence against reactivation of *Herpes simplex* virus in the orolabial epithelia: evaluation of a health claim pursuant to Article 13(5) of Regulation (EC) No 1924/2006

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)

Abstract

Following an application from Granarolo S.p.A., submitted for authorisation of a health claim pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of Italy, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on the scientific substantiation of a health claim related to a low-fat fermented milk and defence against reactivation of *Herpes simplex* virus (HSV) in the orolabial epithelia. The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence. The food which is the subject of the health claim, a low-fat fermented milk with a combination of fructo-oligosaccharides and live *Lactobacillus rhamnosus* GG (ATCC 53103), *Streptococcus thermophilus* (Z57) and *Lactobacillus delbrueckii* subsp. *Bulgaricus* (LB2), is sufficiently characterised. Defence against reactivation of HSV in the orolabial epithelia is a beneficial physiological effect. The Panel considers that no conclusions can be drawn from three out of the four human intervention studies, which were provided by the applicant, for the scientific substantiation of the claim. In weighing the evidence, the Panel took into account that one human intervention study from which scientific conclusions can be drawn does not show a consistent effect of daily consumption of the low-fat fermented milk on the reduction in incidence of *herpes labialis* (HL) lesions after ultraviolet B (UVB) exposure and that no convincing evidence was provided for a mechanism by which the low-fat fermented milk could contribute to the defence against reactivation of HSV in the orolabial epithelia. The Panel concludes that a cause and effect relationship has not been established between the consumption of the low-fat fermented milk, which is the subject of the health claim, and defence against reactivation of HSV in the orolabial epithelia.

Keywords: low-fat fermented milk, LGG, fructo-oligosaccharides, *Herpes simplex* virus, health claims

Requestor: Competent Authority of Italy following an application by Granarolo S.p.A

Question number: EFSA-Q-2015-00488

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Panel members: Jean-Louis Bresson, Barbara Burlingame, Tara Dean, Susan Fairweather-Tait, Marina Heinonen, Karen Ildico Hirsch-Ernst, Inge Mangelsdorf, Harry McArdle, Androniki Naska, Monika Neuhäuser-Berthold, Grażyna Nowicka, Kristina Pentieva, Yolanda Sanz, Alfonso Siani, Anders Sjödin, Martin Stern, Daniel Tomé, Dominique Turck, Hendrik Van Loveren, Marco Vinceti and Peter Willatts

Competing interests: In line with EFSA's policy on declarations of interest, the Panel member Anders Sjödin did not participate in the development and adoption of this scientific opinion.

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Summary

Following an application from Granarolo S.p.A., submitted for authorisation of a health claim pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of Italy, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on the scientific substantiation of a health claim related to a low-fat fermented milk and defence against reactivation of *Herpes simplex* virus (HSV) in the orolabial epithelia.

The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence. The application included a request for the protection of proprietary data.

The general approach of the NDA Panel for the evaluation of health claim applications is outlined in the European Food Safety Authority (EFSA) general guidance for stakeholders on health claim applications and the guidance on the scientific requirements for health claims related to the immune system, the gastrointestinal tract and defence against pathogenic microorganisms.

The food that is the subject of the health claim is a low-fat fermented milk with a combination of fructo-oligosaccharides (FOS) and live *Lactobacillus rhamnosus* GG (LGG; ATCC 53103), *Streptococcus thermophilus* (Z57) and *Lactobacillus delbrueckii* subsp. *Bulgaricus* (LB2). The Panel considers that the low-fat fermented milk, the food which is the subject of the health claim, is sufficiently characterised.

The claimed effect proposed by the applicant is 'helps to reduce recurrence of lip cold sores caused by *Herpes simplex* virus infection' and the target population proposed by the applicant is the 'general healthy population'. Taking into account the EFSA guidance for claims on the immune system, the Panel considers that the claimed effect relates to the defence against reactivation of HSV in the orolabial epithelia, which virus may cause the occurrence of lip cold sores. The incidence of *herpes labialis* (HL) lesions at the end of the study is the appropriate outcome variable to assess this claimed effect. The Panel considers that defence against reactivation of HSV in the orolabial epithelia is a beneficial physiological effect.

The applicant provided four human intervention studies as being pertinent to the health claim.

Two of these studies reported faecal counts of LGG and bifidobacteria following the consumption of a yoghurt added with/without LGG and FOS or fermented milks containing several bacterial strains. The Panel considers that no conclusions can be drawn from these studies on an effect of the low-fat fermented milk, which is the subject of the health claim, on incidence of HL lesions.

Two unpublished reports were provided by the applicant on human intervention studies on the effect of the low-fat fermented milk, which is the subject of the health claim, on incidence of HL lesions. In these studies, participants were randomised to consume the low-fat fermented milk or placebo for 35 or 126 days, respectively. After 19 days of consumption of the study products, participants were exposed to ultraviolet radiation (UVR). At different time points after UV exposure, participants had clinical examinations to ascertain recurrence and progression of HL lesions.

With respect to the first unpublished study, owing to the inconsistent information submitted by the applicant in reply to requests for clarifications during the evaluation procedure, the information on this study was considered insufficient to allow a full scientific evaluation. The Panel considers that no conclusions can be drawn from this study for the scientific substantiation of the claim.

With respect to the second unpublished study, the Panel notes the inconsistency of the results between the intention-to-treat (ITT) and per protocol (PP) analyses, and that no explanations for the different results in the two analyses have been provided. The Panel considers that this study does not show a consistent effect of daily consumption of the low-fat fermented milk, which is the subject of the health claim, on the reduction in incidence of HL lesions after UVB exposure.

The applicant indicated that the low-fat fermented milk could exert the claimed effect by stimulation of the immune system which may affect the HSV latency reactivation. In this respect, the two unpublished studies, which were described above, investigated the effect of the low-fat fermented milk on immunological parameters. The Panel considers that these studies did not show a consistent effect of daily consumption of the low-fat fermented milk on immunological parameters. The applicant also provided *in vitro* and animal studies in support of a mechanism by which the low-fat fermented milk could exert the claimed effect.

The Panel considers that the human, animal and *in vitro* studies submitted by the applicant provided no convincing evidence for a mechanism by which the low-fat fermented milk, which is the subject of the health claim, could exert the claimed effect.

In weighing the evidence, the Panel took into account that one human intervention study from which scientific conclusions can be drawn does not show a consistent effect of daily consumption of the low-fat fermented milk on the reduction in incidence of HL lesions after UVB exposure, and that no

convincing evidence was provided for a mechanism by which the low-fat fermented milk could contribute to the defence against reactivation of HSV in the orolabial epithelia.

The Panel concludes that a cause and effect relationship has not been established between the consumption of the low-fat fermented milk, which is the subject of the health claim, and defence against reactivation of HSV in the orolabial epithelia.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Regulation (EC) No 1924/2006[1] harmonises the provisions that relate to nutrition and health claims, and establishes rules governing the Community authorisation of health claims made on foods. As a rule, health claims are prohibited unless they comply with the general and specific requirements of this Regulation, are authorised in accordance with this Regulation, and are included in the lists of authorised claims provided for in Articles 13 and 14 thereof. In particular, Article 13(5) of this Regulation lays down provisions for the addition of claims (other than those referring to the reduction of disease risk and to children's development and health) which are based on newly developed scientific evidence, or which include a request for the protection of proprietary data, to the Community list of permitted claims referred to in Article 13(3).

According to Article 18 of this Regulation, an application for inclusion in the Community list of permitted claims referred to in Article 13(3) shall be submitted by the applicant to the national competent authority of a Member State, which will make the application and any supplementary information supplied by the applicant available to the European Food Safety Authority (EFSA).

1.2. Interpretation of the Terms of Reference

EFSA is requested to evaluate the scientific data submitted by the applicant in accordance with Article 16(3) of Regulation (EC) No 1924/2006. On the basis of that evaluation, EFSA will issue an opinion on the scientific substantiation of a health claim related to: low-fat fermented milk and defence against reactivation of *Herpes simplex* virus (HSV) in the orolabial epithelia.

The present opinion does not constitute, and cannot be construed as, an authorisation for the marketing of low-fat fermented milk, a positive assessment of its safety nor a decision on whether low-fat fermented milk is, or is not, classified as a foodstuff. It should be noted that such an assessment is not foreseen in the framework of Regulation (EC) No 1924/2006.

It should also be highlighted that the scope, the proposed wording of the claim, and the conditions of use as proposed by the applicant may be subject to changes, pending the outcome of the authorisation procedure foreseen in Article 18(4) of Regulation (EC) No 1924/2006.

2. Data and methodologies

2.1. Data

2.1.1. Information provided by the applicant

2.1.1.1. Food as stated by the applicant

According to the applicant, the food for which the health claim is made is a low-fat fermented milk with a combination of fructo-oligosaccharides (FOS) and live *Lactobacillus rhamnosus* GG (LGG; ATCC 53103), *Streptococcus thermophilus* (Z57) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (LB2).

2.1.1.2. Health relationship as claimed by the applicant

According to the applicant, the claimed effect is 'helps to reduce recurrence of lip cold sores caused by *Herpes simplex* virus infection in healthy susceptible individuals'.

Herpes simplex virus type 1 infects mainly the oropharyngeal mucosa causing *Herpes simplex labialis* or *Herpes labialis* (HL) which manifests in the form of skin lesions commonly known as cold sores.

The outcome variable proposed by the applicant for the assessment of the health claim is the evaluation of the number of subjects who presented lesions in the region of the lips (lip cold sores) at different time points after ultraviolet (UV) exposure.

2.1.1.3. Mechanism by which the food could exert the claimed effect as proposed by the applicant

The applicant indicated that the mechanism by which the low-fat fermented milk could exert the claimed effect involves the stimulation of the immune system. The applicant also referred to *in vitro*

[1] Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. OJ L 404, 30.12.2006, p. 9–25.

studies which indicated that *Lactobacillus rhamnosus* GG (ATCC 53103) could promote survival of epithelial cells and induce transcription of inflammatory and innate immune response genes.

2.1.1.4. Wording of the health claim as proposed by the applicant

The applicant has proposed the following wording for the health claim: 'Consumption of low-fat fermented milk with a combination of fructo-oligosaccharides (FOS) and live *Lactobacillus rhamnosus* GG (ATCC 53103), *Streptococcus thermophilus* (Z57) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (LB2) helps to reduce recurrence of lip cold sores caused by *Herpes simplex* virus infection in healthy susceptible individuals'.

2.1.1.5. Specific conditions of use as proposed by the applicant

The target population proposed by the applicant is the general healthy population.

The applicant has proposed a daily consumption of 90 g of the low-fat fermented milk at breakfast. The low-fat fermented milk, which contains 89% of milk, could reasonably be consumed as part of a balanced diet as a portion of dairy product.

2.1.2. Data provided by the applicant

Health claim application on 'consumption of *Lactobacillus rhamnosus* GG (ATCC 53103) and fructo-oligosaccharides (FOS) helps to reduce recurrence of lip cold sores caused by *Herpes simplex* virus infection in healthy susceptible individuals' pursuant to Article 13.5 of Regulation 1924/2006, presented in a common and structured format as outlined in the Scientific and technical guidance for the preparation and presentation of applications for authorisation of health claims (EFSA NDA Panel, 2011).

As outlined in the General guidance for stakeholders on health claim applications, it is the responsibility of the applicant to provide the totality of the available evidence (EFSA NDA Panel, 2016a).

This health claim application includes a request for the protection of proprietary data in accordance with Article 21 of Regulation (EC) No 1924/2006 for two unpublished study reports (Broccoletti et al., 2009; La Placa et al., 2014) and for data on the composition, manufacturing process and stability of the low-fat fermented milk.

2.2. Methodologies

The general approach of the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) for the evaluation of health claim applications is outlined in the EFSA general guidance for stakeholders on health claim applications (EFSA NDA Panel, 2016a).

The scientific requirements for health claims related to the immune system, the gastrointestinal tract and defence against pathogenic microorganisms are outlined in the specific EFSA guidance (EFSA NDA Panel, 2016b).

3. Assessment

3.1. Characterisation of the food

The food that is the subject of the health claim is a low-fat fermented milk with a combination of FOS and live *Lactobacillus rhamnosus* GG (ATCC 53103), *Streptococcus thermophilus* (Z57) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (LB2).

The strain LGG has been identified and characterised at species and strain level using both phenotypic and genotypic methods. The Panel notes that the culture collection number from the American Type Culture Collection (ATCC 53103) is given. The genome sequence of LGG has been published by Kankainen et al. (2009).

FOS are produced by partial enzymatic hydrolysis of chicory inulin. FOS consist of a mixture of oligosaccharides which are composed of fructose units linked together by $\beta(2\text{–}1)$ linkages and mainly terminate with glucose unit. The total number of fructose or glucose units (degree of polymerisation) of oligofructose ranges mainly between 2 and 8.

Information on the genome sequence of *S. thermophilus* (Z57) and *L. delbrueckii* subsp. *bulgaricus* (LB2) was provided by the applicant.

The composition of a daily serving (90 g) of the fermented milk has been provided: 1.71 g of fat, 9.63 g of carbohydrates, 2.43 g of proteins, 1.8×10^9 colony forming unit (cfu) of LGG, 2.79 g of FOS, 72×10^9 cfu of *S. thermophilus* and 0.9×10^9 cfu of *L. delbrueckii* subsp. *bulgaricus*.

Information pertaining to the manufacturing process and stability tests has been provided by the applicant.

The Panel considers that the low-fat fermented milk with a combination of FOS and live LGG, *S. thermophilus* (Z57) and *L. bulgaricus* (LB2), the food which is the subject of the health claim, is sufficiently characterised.

3.2. Relevance of the claimed effect to human health

The claimed effect proposed by the applicant is 'helps to reduce recurrence of lip cold sores caused by *Herpes simplex* virus infection'. The target population proposed by the applicant is the 'general healthy population'.

The clinical outcome proposed by the applicant to evaluate the claimed effect is the between-group difference in the number of participants who presented *herpes labialis* (HL) lesions at different time points after UV exposure from human intervention studies.

Taking into account the EFSA guidance for claims on the immune system (EFSA NDA Panel, 2016b), the Panel considers that the claimed effect relates to the defence against reactivation of HSV in the orolabial epithelia, which virus may cause the occurrence of lip cold sores. As indicated in this guidance, health claims related to defence against pathogens (such as defence against reactivation of HSV) can be substantiated by human intervention studies which investigate outcomes related to infections (e.g. incidence, severity and/or duration of symptoms). In the context of this health claim application, the Panel considers that between-group differences in the incidence of HL lesions at the end of the study (i.e. total number of participants who experienced at least one HL lesion from UV radiation (UVR) exposure out of those free of lesions at the time of UVR exposure) is the appropriate outcome variable to assess this claimed effect.

The Panel considers that defence against reactivation of HSV in the orolabial epithelia is a beneficial physiological effect.

3.3. Scientific substantiation of the claimed effect

The applicant performed a literature search in PubMed and US National Institutes of Health clinical trial database using an extensive list of keywords.

The applicant provided four human intervention studies as being pertinent to the health claim (Zanini et al., 2007; Broccoletti et al., 2009; Granata et al., 2013; La Placa et al., 2014).

The double-blind, randomised, placebo-controlled study by Granata et al. (2013) reported faecal counts of LGG and bifidobacteria following consumption of a yoghurt added with or without LGG and FOS. The cross-over, single-blind, no-randomised study by Zanini et al. (2007) reported faecal counts of lactobacilli and bifidobacteria following consumption of fermented milks containing several bacterial strains. The Panel considers that no conclusions can be drawn from these studies on an effect of the low-fat fermented milk, which is the subject of the health claim, on incidence of HL lesions.

3.3.1. Human intervention studies on the effect of the low-fat fermented milk on incidence of HL lesions

The applicant provided two unpublished reports on human intervention studies on the effect of the low-fat fermented milk, which is the subject of the health claim, on incidence of HL lesions.

In the placebo-controlled, single-centre study by Broccoletti et al. (2009), 78 adults were randomised to consume daily 90 g of fermented milks for 35 days: the fermented low-fat milk which is the subject of the health claim (LGG plus FOS group; n = 18, which included 10 females); a low-fat milk fermented with LGG, *L. delbrueckii* subsp. *bulgaricus*, and *S. thermophilus*, but without FOS (LGG group; n = 21, which included 12 females); a low-fat milk fermented with *L. delbrueckii* subsp. *bulgaricus*, *S. thermophilus* and added with FOS (FOS group; n = 19, which included nine females); or placebo (n = 20, which included 10 females). The placebo consisted of a low-fat milk, without LGG and FOS, which was fermented with *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*, and then pasteurised. Randomisation was stratified by age and gender.

The amount of FOS and LGG per bottle was 2.79 g and 2×10^9 cfu, respectively, whereas the amount of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* was 72×10^9 cfu and 0.9×10^9 cfu, respectively. The identity of the study products were blinded to participants, support staff members and investigators. The clinical study protocol was approved by the Ethical Committee of Molinette Hospital in Turin (Italy).

Individuals without HL lesions at the time of recruitment, with no more than three HL episodes after exposure to sunlight in the previous year and with Fitzpatrick skin type from I to IV were enrolled in this study. Individuals were excluded if they had HL episodes or if they consumed 'probiotic products' in the month prior to the study; or if they were under medication which could interfere with the immune response (e.g. acyclovir). Concomitant medications and compliance were recorded in diaries by participants. The Panel noted that some participants had HL lesions at screening (n = 1 per group) and at baseline (i.e. randomisation) (n = 2 in the placebo and LGG plus FOS groups; n = 1 in the LGG group). Upon EFSA's request for clarification on this point, the applicant indicated that individuals with HL lesions at screening were allowed to participate in the study as their HL episodes were considered by the investigator to be at a late and ending stage. The applicant also indicated the 'HL lesions at baseline' was not an exclusion criterion in this study.

All participants consumed placebo mini-drinks for 14 days and then they were randomised to consume the study products (fermented milks with LGG plus FOS, only LGG, only FOS or placebo) for 35 days. After 19 days of consumption of the study products, participants were exposed to UVR, which was determined through minimal erythema dose (MED). No rationale was provided for giving UVR amounts that related to the MED, rather than a standard dose of UVR.

After 2, 9 and 16 days from UVR exposure, participants had clinical examinations to ascertain recurrence and progression of HL lesions. At each visit, the following parameters were assessed: lesion development (by classification of lesion stage and photos), duration (i.e. time to healing defined as either loss of the hard crust or return to normal skin) and lesion size (as a product of the length and the width of the lesion). HL signs and symptoms (i.e. dimension, crust, swelling, tenderness) and pain were self-assessed on a 0–4 score scale and visual analogue scale (VAS), respectively. At baseline and at the end of the study, immunological parameters were measured and quality of life was assessed through a SF-36 questionnaire.

Between-group difference in the number of participants with HL lesions at different time points (i.e. at 2, 9 and 16 days after UVR exposure) was the primary outcome of this study.

It is reported in the study that a sample size of 80 participants (i.e. 20 per group) was needed to yield a minimum of 83% statistical power and two-sided 5% significance level.

Fisher's exact test was used to analyse differences in the incidence of HL lesions, new lesions and lesion characteristics among groups. The analysis of variance (ANOVA) was used to analyse differences in pain and quality of life questionnaire among groups; whereas the Wilcoxon rank sum test was used to compare the differences in immune parameters among groups. All tests were two-sided and the significance was set at 5% level. For unpaired comparisons, the significance was set at the 0.008 level (Bonferroni's method).

Statistical analyses of the outcomes investigated were presented for the intention-to-treat (ITT) population and the per protocol (PP) population (i.e. participants who did not violate the protocol and who did not present HL lesions at the time of UVR exposure). The last observation carried forward method was used for imputing missing values in the ITT analysis.

The ITT and PP populations consisted of 78 and 71 participants, respectively. Seven participants were not included in the PP population owing to HL lesions present on the day of the UVR exposure (n = 1 in the placebo group and n = 2 in each of the remaining groups).

At baseline, there were no statistically significant differences between groups in terms of gender, age, body mass index (BMI), human leukocyte antigen, UVR exposure time and compliance, which ranged between 99.5% and 100%.

The between-group differences in the number of participants with HL lesions were presented at different time points (i.e. at 2, 9 and 16 days after UVR exposure) for the ITT and PP population. However, the Panel considers that between-group differences in the incidence of HL lesions at the end of the study (i.e. total number of participants who experienced at least one HL lesion from UVR exposure out of those free of lesions at the time of UVR exposure) is the appropriate outcome variable to assess the claimed effect. Therefore, the applicant was requested to provide the incidence of HL lesions for the ITT and PP population. The Panel notes that the additional information submitted by the applicant in reply to these requests is inconsistent and insufficient to allow a full scientific evaluation of this study (e.g. the number of participants in the study groups in the ITT and PP populations did not match the number of participants declared in the study report; the incidence calculated for some study groups did not match the number of individuals with at least one HL lesion reported in those groups; in a second analysis provided, the incidence in the control group was calculated as the number of lesions, rather than as the number of participants with at least one lesion, out of the number of

subjects at the time of the UVR exposure). The Panel considers that no conclusions can be drawn from this study for the scientific substantiation of the claim.

In the placebo-controlled, double-blind, multicentre study by La Placa et al. (2014), 157 adults were randomised to consume daily 90 g of the low-fat fermented milk which is the subject of the health claim (n = 78, which included 65 females) or placebo (n = 79, which included 59 females) for 126 days. The placebo consisted of low-fat milk, without LGG and FOS, which was fermented with *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*, and then pasteurised.

The amount of FOS and LGG per bottle was 2.79 g and 2 x 10^9 cfu, respectively, whereas the amount of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* was 72 x 10^9 cfu and 0.9 x 10^9 cfu, respectively. The clinical study protocol was approved by the Ethical Committee of Azienda Ospedaliero-Universitaria of Bologna (Italy).

Individuals without HL lesions at the time of recruitment and at baseline, with a history of recurrent HL (two to four episodes) after exposure to sunlight in the previous year and with Fitzpatrick skin type I–IV were enrolled in this study. Individuals were excluded if they were under medication which could interfere with the immune response (e.g. acyclovir). Concomitant medications and compliance were recorded in diaries by participants.

All participants consumed placebo mini-drinks for 14 days and then they were randomised to consume the study products for 126 days. After 19 days of consumption of the study products, participants were exposed to UVB radiation, which was determined through MED. No rationale was provided for giving UVB amounts that related to the MED, rather than a standard dose of UVB.

After 2, 9, 16 and 107 days (end of the study) from UVB exposure, participants underwent clinical examinations to ascertain the recurrence and progression of HL lesions. At each visit, the following parameters were assessed: lesion development (by classification of lesion stage and photos), duration (i.e. time to healing defined as either loss of the hard crust or return to normal skin) and lesion size (longest measure taken). HL signs and symptoms (i.e. crust, swelling, tenderness, overall severity) and pain were self-assessed on a 0–4 scores scale and VAS scale, respectively. At randomisation, after 16 and 107 days from UVB exposure antibody concentrations were measured and quality of life was assessed through a SF-36 questionnaire.

Between-group differences in the number of participants with/without HL lesions after 16 days from UVB exposure was the primary outcome of this study.

A sample size of 152 participants was determined to detect a 30% difference in the incidence of HL lesions between group (power 90%; two-sided 5% significance level).

Fisher's exact test was used to analyse differences in the incidence of HL lesions between groups. Student's t test was used to compare duration, and lesion dimensions between groups. Wilcoxon Rank Sum test was used to analyse immunological parameters.

All participants who consumed at least one dose of intervention or placebo were included in the ITT population (n = 78 in the intervention and n = 79 in the placebo groups, respectively). The PP population consisted of 59 participants in the intervention group and 57 participants in the placebo group. The PP population excluded participants who developed HL before the UVB exposure (n = 6 in both groups), who had protocol violations (n = 7 in the intervention and n = 6 in the placebo groups, respectively) or discontinued the study (n = 6 in the intervention and n = 10 in the placebo groups, respectively).

Statistical analyses of the outcomes investigated were presented for the ITT and PP population. Upon EFSA's request for clarifications, the applicant indicated that all available data were considered for the ITT statistical analysis and no method was used to impute missing data.

At baseline, no statistically significant differences between groups were reported in terms of gender, age, BMI, Fitzpatrick skin type. Compliance to the study products was not statistically significant different between groups (81.4% in the intervention and 75.5% in the placebo groups, respectively).

The between-group differences in the number of subjects with HL lesions, which also included the relapses of HL on the same subject, were presented for the ITT population, with a confirmatory analysis performed in the PP population. In the ITT population, there was no statistically significant between-group difference in the number of subjects with HL lesions after 16 days from UVB exposure. In the PP population, the number of subjects with HL lesions was lower in the intervention group as compared with the placebo group after 16 days from UVB exposure (number of participants with HL lesions: n = 8 in the intervention and n = 17 in the placebo groups, respectively; p = 0.042).

In the ITT population, there was no statistically significant between-group difference in the total number of participants with HL lesions at the end of the study (i.e. after 107 days from UVB exposure), whereas the study reported a statistically significant reduction in the total number of

participants with HL lesions in the intervention group as compared with the placebo group in the PP population (n = 13 in the intervention and n = 25 in the placebo groups, respectively; p = 0.02).

In the PP population, there were no statistically significant between-group differences in the other parameters investigated at the end of the study (i.e. time to healing, lesion size, self-assessed scores on signs and symptoms of HL lesions and self-assessed scores in the quality of life questionnaire). The authors indicated that a statistically significant difference was observed in the self-assessed average scores of pain between groups.

The Panel notes the inconsistency of the results between the ITT and PP analyses. The Panel also notes that no explanations for the different results in the two analyses have been provided. The Panel considers that this study does not show a consistent effect of daily consumption of the low-fat fermented milk, which is the subject of the health claim, on the reduction in incidence of HL lesions after UVB exposure.

3.3.2. Studies on the mechanism by which the low-fat fermented milk could exert the claimed effect

The applicant indicated that the low-fat fermented milk, which is the subject of the health claim, could exert the claimed effect by stimulation of the immune system which may affect the HSV latency reactivation. In this respect, the two unpublished studies which were described in the previous section (Broccoletti et al., 2009; La Placa et al., 2014) investigated the effect of the low-fat fermented milk on immunological parameters.

The study by Broccoletti et al. (2009) reported a statistically significant increase in HSV-specific immunoglobulin 3 (IG3) levels in the low-fat fermented milk group as compared to the placebo group (p = 0.0001); whereas there was no statistically significant difference in HSV-specific IG1 or IG4 levels between groups. This study also reported a statistically significant decrease in interferon-γ (IFN-γ) levels in the low-fat fermented milk group as compared to the placebo group (p = 0.003). A statistically significant increase in '% PMN that phagocytose candida' and in '% PMN that kill candida' was observed in the low-fat fermented milk group as compared to the placebo group (p = 0.008 and 0.0001, respectively).

This study did not report any other statistically significant differences between the low-fat fermented milk and the placebo groups in the other parameters investigated (i.e. cytokines interleukin-2 and interleukin-10; immune phenotypes (CD4, CD8, CD4/CD25, CD8/CD25, CD4/CD45RO, CD8/CD45RO, CD8/CD38/CD45RO); NK activity – % of dead target cells; pentamers – % of positive cells; IFN-γ ELISPOT phenotype).

The study by La Placa et al. (2014) reported a statistically significant between-group difference in CXCL10 levels at day 16 after UVB exposure in a subgroup of participants with HL lesions (p = 0.006); whereas no other statistically significant between-group differences were reported in any of the other immunological parameters investigated (i.e. levels of IgG1, IgG3, IgG4, NK activity, CXCL10 at day 16 after UVB exposure and at the end of the study).

The Panel considers that the studies by Broccoletti et al. (2009) and La Placa et al. (2014) did not show a consistent effect of daily consumption of the low-fat fermented milk, which is the subject of the health claim, on immunological parameters.

The applicant also provided *in vitro* and animal studies in support of a mechanism by which the low-fat fermented milk could exert the claimed effect. The Panel notes that some of these studies were carried out with bacterial strains other than those in the low-fat fermented milk that is the subject of the health claim.

The *in vitro* studies by Yan and Polk (2002) and Tao et al. (2006) indicated that LGG promoted the survival of intestinal epithelial cells by the inhibition of proapoptotic p38/mitogen-activated protein kinase. The applicant hypothesised that LGG could counteract the activation of the p38 signalling apoptotic pathways, which were suggested to be stimulated by HSV-1 proteins (Gillis et al., 2009).

The *in vitro* study by Miettinen et al. (2012) indicated that LGG induced an alteration of gene expression profiles and an increase in the production of interleukin-1β (IL-1β) in human primary macrophages. Considering that HSV-1 appears to prevent secretion of IL-1β in the intracellular space (Milora et al., 2014), the applicant hypothesised that LGG helps to re-establish the concentration of IL-1β.

Liu et al. (2013) investigated the effect of LGG on diarrhoea episodes, histopathology of the ileum and serum cytokine responses in a pig model of virulent human rotavirus (HRV) infection.

Capitán-Cañadas et al. (2014) reported the effect of non-digestible oligosaccharides, including FOS, on cytokine secretion in primary rat monocytes and in human peripheral blood monocytes.

The Panel considers that the human, animal and *in vitro* studies submitted by the applicant provided no convincing evidence for a mechanism by which the low-fat fermented milk, which is the subject of the health claim, could exert the claimed effect.

3.3.3. Weighing of the evidence

In weighing the evidence, the Panel took into account that one human intervention study from which scientific conclusions can be drawn does not show a consistent effect of daily consumption of the low-fat fermented milk on the reduction in incidence of HL lesions after UVB exposure and that no convincing evidence was provided for a mechanism by which the low-fat fermented milk could contribute to the defence against reactivation of HSV in the orolabial epithelia.

The Panel concludes that a cause and effect relationship has not been established between the consumption of the low-fat fermented milk, which is the subject of the health claim, and defence against reactivation of HSV in the orolabial epithelia.

4. Conclusions

On the basis of the data presented, the Panel concludes that:

- the low-fat fermented milk with a combination of FOS and live LGG, *S. thermophilus* (Z57) and *L. bulgaricus* (LB2), the food which is the subject of the health claim, is sufficiently characterised.
- the claimed effect proposed by the applicant is 'helps to reduce recurrence of lip cold sores caused by *Herpes simplex* virus infection'. The target population proposed by the applicant is the 'general healthy population'. Defence against reactivation of HSV in the orolabial epithelia is a beneficial physiological effect
- a cause and effect relationship has not been established between the consumption of the low-fat fermented milk and defence against reactivation of HSV in the orolabial epithelia.

Steps taken by EFSA

1) Health claim application on 'consumption of *Lactobacillus rhamnosus* GG (ATCC 53103) and fructo-oligosaccharides (FOS) helps to reduce recurrence of lip cold sores caused by *Herpes simplex* virus infection in healthy susceptible individuals' pursuant to Article 13(5) of Regulation (EC) No 1924/2006 (Claim serial No: 0439_IT). Submitted by Granarolo S.p.A., Via Cadriano 27/2, 40127, Bologna, Italy.
2) This application was received by EFSA on 31/8/2015.
3) The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence. The application included a request for the protection of proprietary data.
4) On 9/10/2015, during the validation process of the application, EFSA sent a request to the applicant to provide missing information.
5) On 14/12/2015, EFSA received the missing information as submitted by the applicant.
6) The scientific evaluation procedure started on 16/12/2015.
7) On 20/1/2016, the Working Group on Claims of the NDA Panel agreed on a list of questions for the applicant to provide additional information to accompany the application. The scientific evaluation was suspended on 09/02/2016 and was restarted on 24/2/2016, in compliance with Article 18(3) of Regulation (EC) No 1924/2006.
8) On 25/2/2016, EFSA received the applicant's reply (which was made available to EFSA in electronic format on 24/2/2016).
9) On 16/3/2016, the Working Group on Claims of the NDA Panel agreed on a list of questions for the applicant to provide additional information to accompany the application. The scientific evaluation was suspended on 11/4/2016 and was restarted on 26/4/2016, in compliance with Article 18(3) of Regulation (EC) No 1924/2006.
10) On 28/4/2016, EFSA received the applicant's reply.
11) During its meeting on 28/6/2016, the NDA Panel, having evaluated the data, adopted an opinion on the scientific substantiation of a health claim related to low-fat fermented milk and defence against reactivation of *Herpes simplex* virus in the orolabial epithelia.

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Abbreviations

ANOVA	analysis of variance
ATCC	American Type Culture Collection
BMI	body mass index
CFU	colony forming unit
FOS	fructo-oligosaccharides
HL	*Herpes labialis*
HSR	human rotavirus
HSV	*Herpes simplex* virus
IG	immunoglobulin
IgG	immunoglobulin G
IL-1β	interleukin-1β
ITT	intention-to-treat

LGG	*Lactobacillus rhamnosus* GG (ATCC 53103)
MED	minimal erythema dose
NDA Panel	EFSA Panel on Dietetic Products, Nutrition and Allergies
PMN	polymorphonuclear cell
PP	per protocol
UVR	ultraviolet radiation
VAS	visual analogue scale